

THE BRACTEOLAS IN *AMARANTHUS* (AMARANTHACEAE): THEIR MORPHOLOGY, STRUCTURE, FUNCTION, AND TAXONOMIC SIGNIFICANCE

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ABSTRACT

The morphology and anatomy of bracteoles were surveyed in 20 *Amaranthus* taxa in order to determine their taxonomic significance and role. Three types of bracteoles were distinguished: spinose, foliaceous and membranous. The bracteoles retain the C_4 structure of the leaves, but they differ from the latter by the presence of well-developed sclerenchyma and the arch-shaped Kranz bundle-sheath. Protection and photosynthesis are the two functions of bracteoles inferred from their structure. Based on their structure, foliaceous and membranous bracteoles are considered symplesiomorphic, while spinose bracteoles are interpreted as synapomorphic. The spines present at the base of each leaf in *A. spinosus* L. are metamorphosed bracteoles. The shortness of bracteoles in the grain amaranths (*A. caudatus* L., *A. cruentus* L. and *A. hypochondriacus* L.) was found to be a reliable character, which separates them from their wild relatives (*A. hybridus* L. and *A. powellii* S. Watson). The structure of bracteoles may be significant for recognition of taxa in only a few cases: *A. hybridus* L. and *A. powellii* S. Wats.; *A. powellii* subsp. *powellii* and subsp. *bouchonii* (Thell.) Costea & Carretero and *A. blitum* L. agg.

RESUMEN

La morfología y anatomía de las bracteolas de 20 taxa de *Amaranthus* fueron examinadas para determinar su significado taxonómico y su función. Se diferenciaron tres tipos de bracteolas: espinosas, foliáceas y membranosas. Las bracteolas retienen la estructura C_4 de las hojas, pero difieren de estas últimas por la presencia de un esclerénquima bien desarrollado y en la forma arqueada de la vaina de los haces de tipo Kranz. Las dos funciones de las bracteolas son la protección y la fotosíntesis según se deduce de su estructura. Basándonos en su estructura, las bracteolas foliáceas y membranosas son consideradas como simplesiomórficas, mientras que las bracteolas espinosas son interpretadas como sinapomórficas. Las espinas presentes en la base de cada hoja de *A. spinosus* L. son bracteolas metamorfoseadas. La cortedad de las bracteolas en los amarantos cultivados como pseudocereales (*A. caudatus* L., *A. cruentus* L. y *A. hypochondriacus* L.) fue encontrada como un carácter que los separa de sus parientes silvestres (*A. hybridus* L. y *A. powellii* S. Watson). La estructura de las bracteolas puede ser significativa para el reconocimiento de taxa sólo en algunos casos: *A. hybridus* L. y *A. powellii* S. Wats.; *A. powellii* subsp. *powellii* y subsp. *bouchonii* (Tell.) Costea & Carretero y *A. blitum* agg.

INTRODUCTION

The genus *Amaranthus* is a group of extremely high economical value. Some species are grown as vegetables and pseudocereals (reviewed by Brenner et al. 2000), while others are among the most important weeds of the world (Holm et

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al. 1997). Additionally, some species are widespread ornamentals and they have a potential as forage crops and as a source of red food colorants (Cai et al. 1988). The taxonomical treatment of some species has proven to be problematic (e.g. Costea et al. 2001; Xu & Sun 2001), and it is proposed that characters of bracteoles may bring forward additional data useful for the classification of the species within this genus. Bracts are modified leaves without an axillary bud, associated with the inflorescence or with individual flowers (McCusker 1999). Bracteoles are secondary bracts borne singly (usually in monocotyledons) or in pairs on the pedicel, the calyx or the perianth of a flower (McCusker 1999). In *Amaranthus*, bracteoles should not be mistaken with the tepals. The latter are the units of perianth, which is not differentiated in a calyx and a corolla (McCusker 1999). As Brenan (1961) wrote: "the size and shape of longer bracteoles in the inflorescence, and particularly their length relative to the flowers they subtend, are of great importance in distinguishing several species of the genus *Amaranthus*." In the difficult *A. hybridus* aggregate, the morphology of bracteoles has been used to differentiate grain amaranths (*Amaranthus caudatus*, *A. cruentus* and *A. hypochondriacus*) from their wild relatives (*A. hybridus* subsp. *quitensis*, *A. hybridus* subsp. *hybridus* and *A. powellii* subsp. *powellii*) (reviewed by Costea et al. 2001). The length of bracteoles has also traditionally been used to classify the infraspecific variability of some species such as *A. retroflexus* (e.g., Thellung 1914). We have proposed to reevaluate the bracteoles in *Amaranthus* in order to determine their taxonomic significance.

Amaranthus spp. are C₄ plants and, therefore, exhibit the characteristic Kranz anatomy in the leaves (best described in *A. retroflexus* by Fischer & Evert 1982) and cotyledons (Wang et al. 1993). No anatomical study of bracteoles has been published in *Amaranthus*, and it would be interesting to compare their anatomy with that of the leaves. Furthermore, the structure may indicate their function and could provide new relevant data regarding the evolution of the genus.

Amaranthus spinosus is the only species of the genus that exhibits two spines at the base of each leaf, and Thellung (1914) suggested that the two spines are actually metamorphosed bracteoles. Testing this hypothesis was another objective of this study.

MATERIALS AND METHODS

Morphology of female bracteoles was examined both on herbarium and fresh material. Only the bracteoles subtending the flowers during fructification were taken into consideration. The following herbaria collections were surveyed for the nine qualitative characteristics (see below): BH, BP, BPI, BRIT, CAS, CLA, CM, DAO, DAV, DS, DUKE, FLAS, HAM, I, IBE, ISC, LA, LIL, MICH, MIL, MIN, MO, MSC, MT, NA, OAC, OKL, PRH, QFA, RB, RSA, SMU, SOM, TEX, UCR, UMO, US, UTEP, VAB, VDB, VF and WIS. Additionally, bracteoles were collected from the wild flora (mainly from Romania and Spain) and from cultivated accessions

provided by the USDA and Gatersleben germplasm collections (Table 1). Voucher specimens are preserved in the BUAG herbarium collection, except for the USDA accessions, which are preserved in the US collection. For the anatomical study, twenty bracteoles were collected from each plant, and 15–20 different plants for each species were examined. Bracteoles were fixed in formalin-acetic acid-alcohol (5:5:90) (FAA) and embedded in paraffin. Transverse sections were made at 5–7 mm in thickness and stained with Toluidine Blue. The structure of the bracteoles varies along with their length. The most complete region is usually at the base (0.2–0.4 mm) of bracteoles, and characters refer to this region. Drawings were prepared with a Reichart camera lucida.

List of bracteole characters examined (See the results section for additional explanations.):

a) qualitative characters of bracteoles (Table 2)

1. Bracteole type:
 - s, spinose;
 - f, foliaceous;
 - m, membranous;
2. Branching of mid-vein:
 - +, branched;
 - , unbranched;
3. Pattern of mesophyll development along the bracteole's mid-vein:
 - +, uniform, mesophyll more or less uniformly distributed along the mid-vein;
 - , not uniform, mesophyll is more developed in certain regions, usually at the middle or above the middle;
4. Color of the mid-vein (examined on dry material):
 - g, green (usually dark-green);
 - y, yellow to yellowish-brown or reddish;
5. Shape of the median part of bracteoles (vascular tissue + sclerenchyma + mesophyll) as seen in cross-sections (examined only for spinose bracteoles):
 - cir, semicircular;
 - el, semi-elliptic;
 - de, deltoid;
 - tri, trilobate;
6. Spatial pattern of Kranz bundle sheath arrangement (only for bracteoles with branched mid-veins):
 - sep, Kranz sheath separately surrounds each branch of the mid-vein;
 - tog, Kranz sheath encloses all the branches together;
7. Shape of "Kranz cells" as seen in cross-section:
 - ro, rounded or square;
 - pa, palisade like;
8. Bundle sheath:
 - co+, bundle sheath in contact with the lower epidermis at least in some points;
 - co-, additional layers of mesophyll are present between the bundle sheath and the lower epidermis;
9. Mesophyll cells (when present):
 - pa, palisade-like;
 - spo, spongy parenchyma-like;
 - tan, tangentially elongated;

TABLE 1. Provenance of *Amaranthus* taxa (Amaranthaceae) examined for anatomy of bracteoles (Germany* = accessions from Gatersleben).

Taxa	Voucher no. or accession	Provenance
Subgenus <i>Acnida</i> (L.) Aellen ex K.R. Robertson		
<i>A. palmeri</i> S. Wats.	23522 (a-j)	Romania
Subgenus <i>Amaranthus</i> (= section <i>Amaranthus</i>)		
<i>A. caudatus</i> L.	23050 (a-j) (k-s)	Romania Germany*
	Ames 2026	Nepal
	PI 16604	India
	PI 490440	Peru
<i>A. cruentus</i> L.	23037 (a-j) PI 566896 PI 566897 PI 511919	Romania Arizona, USA India Guatemala
<i>A. hypochondriacus</i> L.	22769 (a-j) (k-s) PI 511 721	Romania Germany* Mexico
<i>A. powellii</i> S. Wats. subsp. <i>powellii</i>	22770 (a-j) (k-s)	Romania Spain
<i>A. powellii</i> subsp. <i>bouchonii</i> (Thell.) Costea & Carretero	23041 (a-j) (k-s)	Romania Spain
<i>A. hybridus</i> L. subsp. <i>hybridus</i>	22721 (a-j) (k-s)	Romania Spain
<i>A. hybridus</i> subsp. <i>quitensis</i> (Thell.) Costea & Carretero	22840 (a-j) (k-s)	Romania Germany*
<i>A. spinosus</i> L.	23866 (a-j)	Germany*
<i>A. retroflexus</i> L.	21822 (a-j) (k-s)	Romania Spain
Subgenus <i>Albersia</i> (Kunth) Gren. & Godr. (= Section <i>Blitopsis</i> Dumort.)		
<i>A. albus</i> L.	21800 (a-j) (k-s) Ames 13788	Romania Spain Canada
<i>A. blitum</i> L. subsp. <i>blitum</i>	23049 (a-j) (k-s)	Romania Germany*
<i>A. blitum</i> subsp. <i>oleraceus</i> (L.) Costea	23051(a-s) PI 606 281	Germany* Bangladesh
<i>A. emarginatus</i> Moq. ex Uline & Bray = <i>A. blitum</i> subsp. <i>emarginatus</i> (Moq. ex Uline & Bray) Carretero, Munoz Garmendia & Pedrol	22996 (a-j) (k-s) Ames 23387 Ames 14964	Romania Spain Brazil India
<i>A. viridis</i> L.	23034 (a-j) (k-s)	Romania Spain

TABLE 1. continued

Taxa	Voucher no. or accession	Provenance
<i>A. blitoides</i> S. Wats.	23045 (a–j)	Romania
	(k–s)	Spain
	PI 553 059	Canada
	PI 608 663	USA
<i>A. crispus</i> (Lesp. & Thev.) N. Terraciano	22225 (a–j)	Romania
<i>A. deflexus</i> L.	22228 (a–j)	Romania
	(k–s)	Spain

10. Sclerenchyma (only for bracteoles with branched mid-veins):
 - com, compact, enclosing all the vascular tissue;
 - frag, fragmented, separately accompanying each vein;
11. Base of the wings containing:
 - m+s, with mesophyll + sclerenchyma;
 - m, only with mesophyll;
 - s, only with sclerenchyma;
 - , neither mesophyll nor sclerenchyma is present;
- b) quantitative characters of bracteoles (Table 3)
 1. Length (mm);
 2. Maximum width at the base (mm);
 3. Length of the spinose apex—only for spinose bracteoles (μm);
 4. Angle between wings and the median part;
 5. Thickness at the base (measured on cross-sections) (μm);
 6. Thickness of the collateral bundle or of the main collateral bundle in the case of branched mid-veins (μm);
 7. Thickness of mesophyll (μm);
 8. Thickness of bundle sheath cells (μm);
 9. Ratio: bracteoles length/ perianth length.

RESULTS

In all the species examined, each flower is subtended by two bracteoles (sometimes only one, or up to four) which are more or less modified compared to the leaves. The bracteoles in *Amaranthus* can be classified as follows:

a) *Spinose bracteoles* (Fig. 1, A–F; Fig. 3A).—Are rigid and have a spiny tip. They have a median part that includes the mid-vein surrounded by sclerenchyma and mesophyll, and two membranous lateral parts that we shall name “wings,” due to of their membranous appearance. The sclerenchyma is more developed than the mesophyll, conferring the hard and rigid appearance of bracteoles. As seen in cross-sections, the median part can be semicircular (Fig. 1C, E) semi-elliptic (Fig. 1D), deltoid (Fig. 1B, F) or even trilobed (Fig. 2A) (Table 2). The wings are not arranged in the same plane with the median part. They form a characteristic angle with the median part, which is maintained even in cross-sections (Table 3). Representative species: *Amaranthus powellii* (Fig. 1E).

TABLE 2. Data matrix for qualitative characters in some species of the genus *Amaranthus* (Amaranthaceae). See "material and methods" for the numbered characters; na = not applicable.

Taxa/Character	1	2	3	4	5	6	7	8	9	10	11
<i>A. palmeri</i>	s	+	+	g	de	tog	na	na	spo	com	s, m+s
<i>A. caudatus</i>	s	-	+	y	cir	na	ro	co-	spo	na	m
<i>A. cruentus</i>	s	-	+	y	el	na	ro	co-	spo	na	m+s
<i>A. hypochondriacus</i>	s	-	+	y	cir	na	pa	co-	pa	na	m
<i>A. powellii</i> subsp. <i>powellii</i>	s	-	+	y	cir	na	pa	co-	pa	na	m
<i>A. powellii</i> subsp. <i>bouchonii</i>	s	-	+	y	de	na	ro/pa	co+	spo	na	m+s
<i>A. hybridus</i> subsp. <i>hybridus</i>	s	-	+	g	de	na	ro	co-	tan	no	m+s
<i>A. hybridus</i> subsp. <i>quitensis</i>	s	-	+	g	de	na	ro	co-	tan	no	m+s
<i>A. spinosus</i>	s	+	+	g	dir	tog	na	na	spo	com	s, m+s
<i>A. retroflexus</i>	s	-	+	y	cir/de	na	ro/pa	co+	spo	na	s, m+s
<i>A. albus</i>	s	-	+	y	de/tri	na	ro	co-	pa	na	m
<i>A. blitum</i> subsp. <i>blitum</i>	f/m	-	-	g	na	na	ro	co-	spo	na	na
<i>A. blitum</i> subsp. <i>oleraceus</i>	f	+	-	g	na	sep	ro	co-	spo	sep	na
<i>A. blitum</i> subsp. <i>emarginatus</i>	m	-	-	g	na	na	ro	co+	na-spo	na	na
<i>A. viridis</i>	f/m	-	-	g	na	na	ro	co-	spo	na	na
<i>A. blitoides blitoides</i>	f	+	-	g	na	sep/tog	ro/pa	co-	spo	com	na
<i>A. crispus</i>	m	-	-	g	na	na	ro	co+	na-spo	na	na
<i>A. deflexus</i>	m	-	-	g	na	na	ro	co+	na-spo	na	na

b) *Foliaceous bracteoles* (Fig. 2C-F).—Resemble more a small leaf, and they do not exhibit an obvious differentiation between the median and lateral parts. The mesophyll is more developed than the sclerenchyma, and as a result, the bracteoles are mostly green. The wings are narrow. Representative species: *A. blitoides* (Fig. 2E-F).

c) *Membranous bracteoles* (Fig. 2B).—Resemble the foliaceous bracteoles, but the mesophyll is poorly developed, restricted along the mid-veins, and is often reduced to the one-layered bundle-sheath. Sclerenchyma is also scarcely developed. The rest of the bracteole is thin, and membranous (as in the wings of the spinose bracteoles). Representative species: *A. crispus*. Transitional forms between the foliaceous and the membranous types occur in several species (*A. blitum* subsp. *blitum* and *A. viridis*).

The structure of bracteoles is simple: they have a dorsiventral structure, and they are composed of epidermis, mesophyll, vascular tissue and sclerenchyma. The marginal-meristem activity is intense; its cells proliferate as monolayers

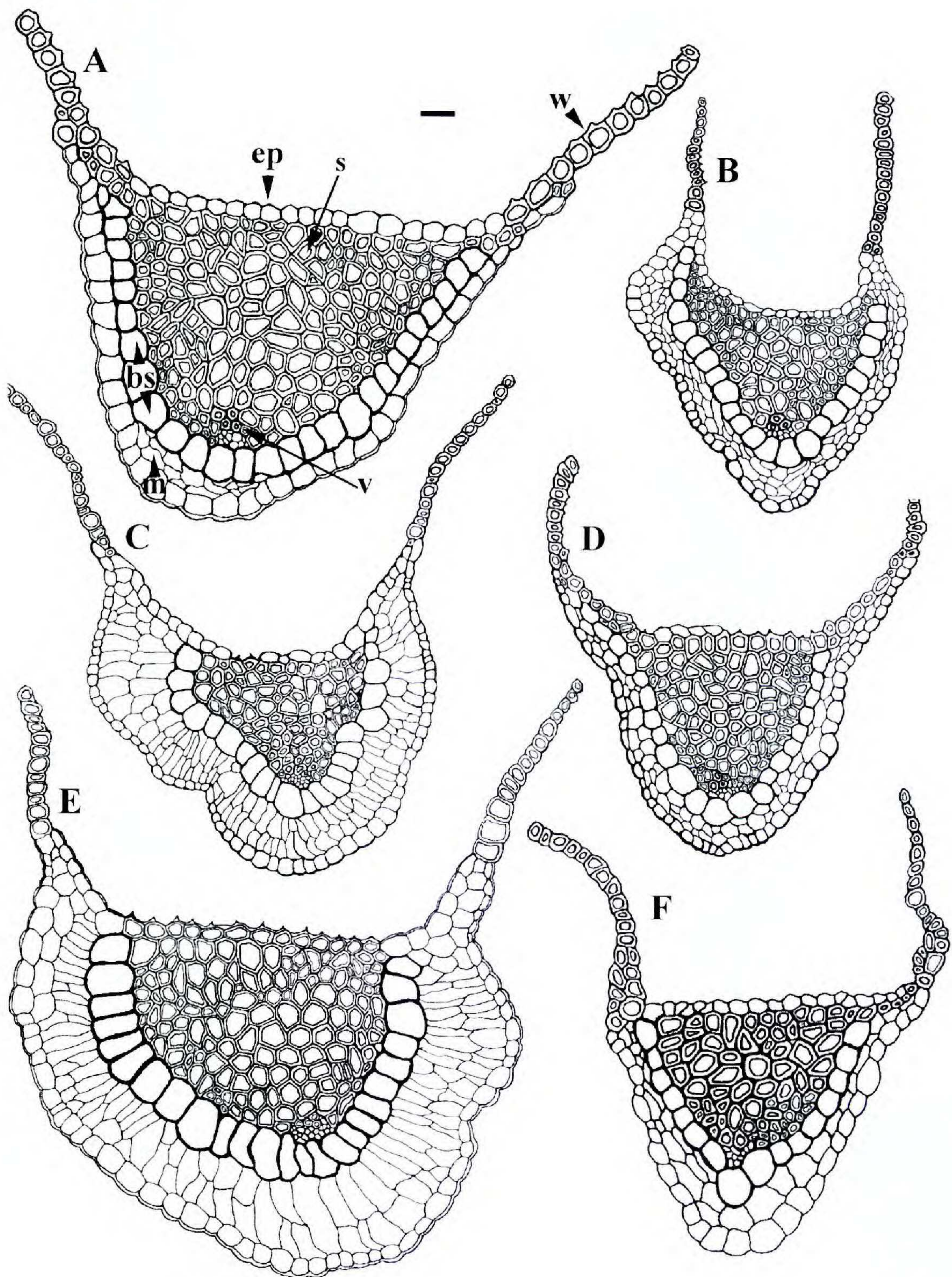


FIG. 1. Structure of spinose bracteoles. **A.** *Amaranthus retroflexus*, **B.** *A. hybridus* (incl. ssp. *quitensis*), **C.** *A. hypochondriacus*, **D.** *A. cruentus*, **E.** *A. powellii* ssp. *powellii*; **F.** *A. powellii* ssp. *bouchonii*. bs-kranz bundle-sheath, ep-epidermis, m-mesophyll, s-sclerenchyma, v-vascular tissue (collateral bundle), w-wing. Scale bar = 20 μ m.

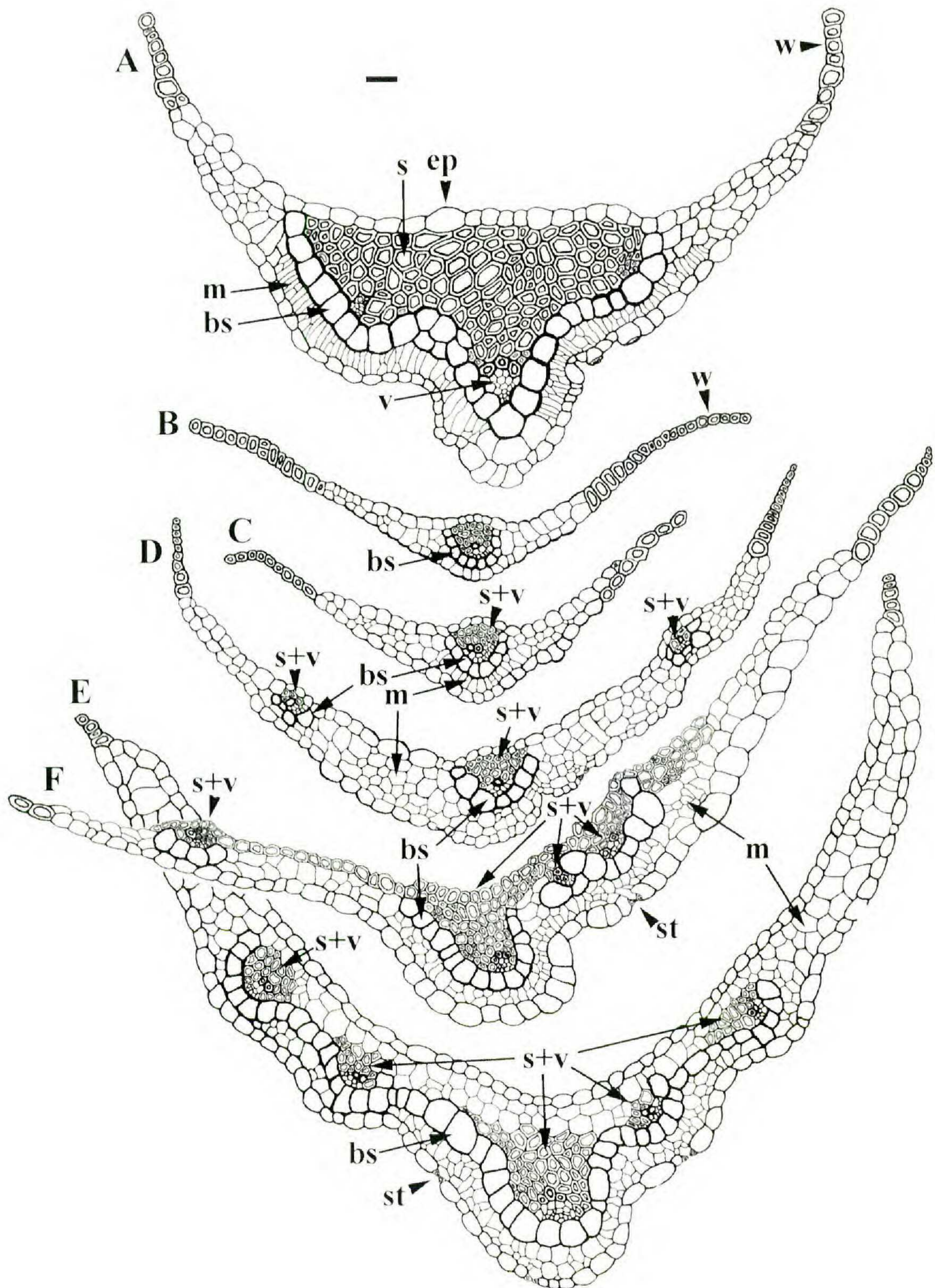


FIG. 2. Structure of bracteoles. **A.** spinose in *Amaranthus albus*, **B.** membranous in *A. blitum* ssp. *emarginatus*, **C–F** foliaceous in: **C.** *A. blitum* ssp. *blitum*, **D.** *A. blitum* ssp. *oleraceus*, **E–F.** *A. blitoides*, **E.** sectioned at the middle, **F.** sectioned at the base. bs-kranz bundle-sheath, ep-epidermis, m-mesophyll, st-stomata, s-sclerenchyma, v-vascular tissue (collateral bundle), w-wing. Scale bar = 20 μ m.

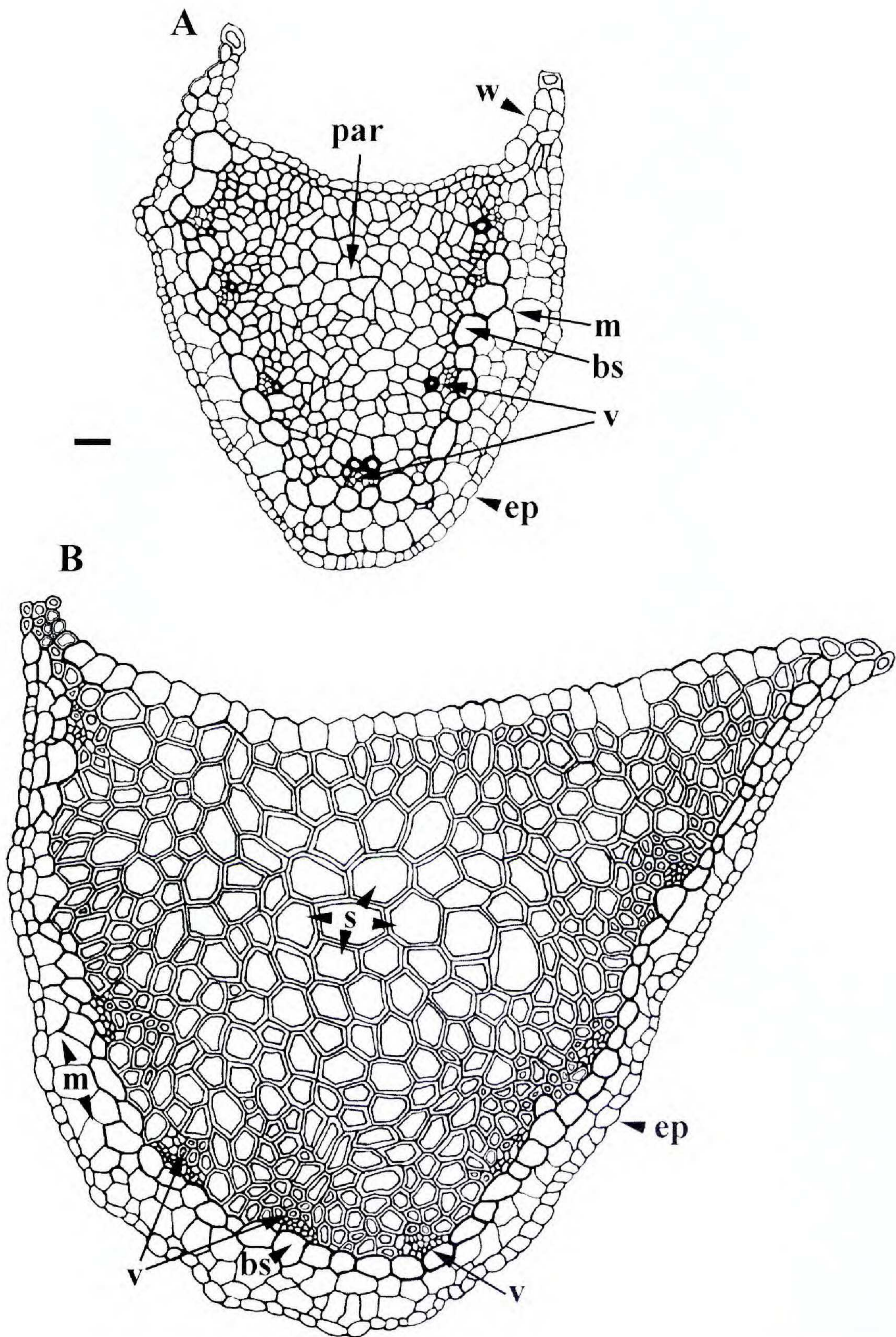


FIG. 3. Structure of spines in *Amaranthus spinosus*. **A.** early stage, bracteole-like, **B.** mature spine. bs-kranz bundle-sheath, ep-epidermis, m-mesophyll, par-parenchyma, s-sclerenchyma, v-vascular tissue (collateral bundle), w-wing. Scale bar = 20 μ m.

TABLE 3. Data matrix for the quantitative characteristics of bracteoles in some *Amaranthus* taxa (Amaranthaceae). See material and methods for numbered characters.

Taxa/Character	1	2	3	4	5	6	7	8	9
<i>A. palmeri</i>	5.5 ± 1	1.4 ± 0.1	2.5 ± 0.5	135 ± 5	320 ± 20	300 ± 25	22.5 ± 2.5	22.5 ± 2.5	1.3–2.1
<i>A. caudatus</i>	3.25 ± 1.75	0.47 ± 0.1	0.67 ± 0.1	137.5 ± 2.5	215 ± 30	155 ± 15	55 ± 5	22.5 ± 2.5	1.2–1.5
<i>A. cruentus</i>	2.25 ± 0.25	0.95 ± 0.1	0.65 ± 0.5	135 ± 5	175 ± 25	125 ± 5	50 ± 10	22.5 ± 2.5	1–1.25
<i>A. hypochondriacus</i>	3.5 ± 0.5	1.2 ± 0.25	1 ± 0.15	135 ± 5	210 ± 10	160 ± 10	100 ± 20	20 ± 15	1.25–1.6
<i>A. powellii</i> subsp. <i>powellii</i>	6.5 ± 1.5	2.4 ± 0.25	2.5 ± 0.5	132.5 ± 7.5	320 ± 20	210 ± 10	100 ± 10	55 ± 5	2–3
<i>A. powellii</i> subsp. <i>bouchonii</i>	4.25 ± 0.75	1.15 ± 0.25	1.2 ± 0.3	127.5 ± 7.5	175 ± 25	122.5 ± 12.5	40 ± 20	22 ± 2.5	2–2.2
<i>A. hybridus</i> subsp. <i>hybridus</i>	3.75 ± 1.15	1.15 ± 0.25	1 ± 0.2	95.5 ± 5	165 ± 15	110 ± 10	45 ± 5	22 ± 2.5	1.2–2
<i>A. hybridus</i> subsp. <i>quitensis</i>	3.75 ± 0.75	1.10 ± 0.23	0.67 ± 0.5	95 ± 5	165 ± 15	110 ± 10	45 ± 5	22 ± 2.5	1.2–2
<i>A. spinosus</i>	3.6 ± 0.9	1.25 ± 0.25	1.02 ± 0.27	105 ± 15	270 ± 30	220 ± 20	71 ± 11	21 ± 2.2	1.5–2
<i>A. retroflexus</i>	5.25 ± 1.25	1.3 ± 0.5	2.17 ± 0.17	135 ± 5	230.5 ± 30.5	203 ± 6	25 ± 25	20 ± 15	1.1–2.8
<i>A. albus</i>	4.2 ± 1.8	1.12 ± 0.22	1 ± 0.2	165 ± 5	240 ± 20	162.5 ± 12.5	76.5 ± 4	20 ± 15	2–2.5
<i>A. blitum</i> subsp. <i>blitum</i>	1.03 ± 0.25	0.75 ± 0.15	-	-	56 ± 6	32 ± 2	32.5 ± 7.5	12.5 ± 2.5	0.6–0.9
<i>A. blitum</i> subsp. <i>oleraceus</i>	1.25 ± 0.35	0.8 ± 0.1	-	-	80 ± 10	37.5 ± 2.5	45 ± 5	16.5 ± 1	0.6–0.9
<i>A. blitum</i> subsp. <i>emarginatus</i>	0.5 ± 0.1	0.45 ± 0.5	-	-	42.5 ± 7.5	26 ± 4	12.5 ± 12.5	10 ± 1.4	0.4–0.7
<i>A. viridis</i>	0.62 ± 0.7	0.5 ± 0.1	-	-	45 ± 5	22.5 ± 2.5	25 ± 2.5	12.5 ± 2.5	0.6–0.9
<i>A. blitoides</i>	2.15 ± 0.15	1.65 ± 0.34	-	-	177.5 ± 12.5	74 ± 12.8	76 ± 5	32.7 ± 2.4	0.8–0.95
<i>A. crispus</i>	1 ± 0.1	0.45 ± 5	-	-	42.5 ± 12.5	30.5 ± 11	10 ± 10	9 ± 1.1	0.6–0.9
<i>A. deflexus</i>	1.15 ± 0.5	0.8 ± 0.1	-	-	52.5 ± 7.5	32.5 ± 2.5	17.5 ± 17.5	12.5 ± 2.5	0.73–0.95

and give rise to the wings. Venation is poor, but the vascular tissue is accompanied by well-developed sclerenchyma. Particularly the lower epidermis may have anomocytic stomata, with three to six neighboring cells surrounding the guard cells. Sparse, multicellular, uniseriate trichomes may occur in all species, especially toward the base of the bracteoles. The vascular tissue consists of a single colateral bundle surrounded by sclerenchyma and a bundle sheath. In some of the species examined—*A. spinosus*, *A. palmeri*, *A. blitoides* and *A. blitum* subsp. *oleraceus*—the mid-vein branches (Fig. 2D–F; Table 2). In all species, the bundle sheath exhibits the “Kranz syndrome” characteristic of the leaves. The fundamental difference is that the bundle-sheath is incomplete, with the cells arranged in an arc around the sclerenchyma. The bundle sheath cells are tightly packed together, they have thicker walls, and their chloroplasts are larger than those of the mesophyll cells. In the species with bracteoles possessing a branched mid-vein, the “Kranz” bundle sheath can separately follow each branch (*A. blitum* subsp. *oleraceus*, Fig. 2D), or it can enclose all the branches together (*A. palmeri* and *A. spinosus*, Fig. 3A). In *A. blitoides* both situations may be encountered in the same bracteole: the Kranz bundle-sheath may be continuous at the base of the bracteoles (Fig. 2E) and fragmented above their middle (Fig. 2F). The mesophyll is homogenous and distributed only along the abaxial face. Its cells, arranged in up to four layers, can be palisade-like (Fig. 1C, E; Fig. 2A), spongy parenchyma-like (e.g. Fig. 1D) or, tangentially elongated (e.g. Fig. 1B) (Table 2). The mesophyll can be uniformly distributed along the mid-vein, or the mesophyll may develop preponderantly along certain regions of the bracteoles (usually in the middle and above). In the first case, the mid-vein morphologically appears “constant,” gradually decreasing along its course towards the apex. In the second case, the mid-vein appears enlarged above the middle of the bracteoles. The sclerenchyma in spinose bracts is strongly developed, compact, engulfing the mid-vein and, when present, its secondary branches (e.g. in *A. spinosus* and *A. palmeri*). In foliaceous and membranous bracts with branched veins, sclerenchyma can be compact or fragmented, separately accompanying each branch (Fig. 2D) (Table 2). The wings have several layers of cells at their base and they are one-layered in the rest. The base of the wings may exhibit only mesophyll cells, only sclerenchyma, mesophyll and sclerenchyma cells or none one of these (Fig. 1; Table 2). The monolayer of wings has thickened cell walls and may contain calcium oxalate crystals.

Origin of spines in Amaranthus spinosus.—This study supports Thellung’s (1914) hypothesis. The two spines present at the base of each leaf are the metamorphosed bracteoles of the first flower within the first dichazial cyme, whose development is suppressed. No floral rudiments were observed in the vicinity of the developing spines which indicates that the suppression is achieved very early in the ontogeny. In an incipient stage of development, spines are similar to bracteoles (Fig. 1A). However, in bracteoles, the development of sclerenchyma

is rapidly completed. The growth of spines involves primarily cell expansion. Sclerosis is delayed until the parenchyma cells enlarge up to five times compared to the parenchyma cells of the bracteoles. Only then is the development of sclerenchyma completed (Fig. 3B). The marginal meristems make at most a limited contribution to the development of the spines, and consequently the wings are reduced or entirely absent (Fig. 3A, B). Mesophyll is present at least towards the base of spines and the vascular supply is similar to that of the bracteoles (Fig. 3B). *Amaranthus spinosus* has a form—f. *inermis* Lautersbach and Schumann—that has no spines, or with spines that are bracteole-like, subtending the first dichazial cyme of each leaf. We believe this form can be regarded as the ancestral type, from which the spiny plants derived. We observed a similar tendency of bracteoles' transformation in some individuals of *A. powellii*. However, the degree of sclerenchyma development never attains the levels observed in *A. spinosus*, and the process is not accompanied by the developmental suppression of the first cyme.

DISCUSSION

Taxonomic significance of bracteoles.—Taking into account the foliar origin of bracteoles, their phylogeny can be speculated upon. The primitive condition in *Amaranthus* is represented by the foliaceous and membranous bracteoles, while the spinose bracteoles can be considered a synapomorphy. Consequently, the most primitive *Amaranthus* species are those classified in the subgenus *Albersia*, while those from the subgenus *Amaranthus* are more evolved. This hypothesis is also supported by the evolution of other characteristics such as the dehiscence-indehiscence of fruits (Costea et al. in press) and the vascular system (Costea & DeMason 2001).

Based on the populations and accessions used, only a few of the taxonomically difficult species with spinose and foliaceous bracteoles could be separated using anatomical characteristics. Furthermore, such characteristics are even less significant in the species with membranous bracteoles, due to their extreme reduction. The anatomic differences between grain amaranths and their wild relatives are minute (Table 2, 3; Fig. 1), and it is possible that these differences will be found to be even less significant when more accessions are examined. There are no differences between *A. hybridus* subsp. *hybridus* and *A. hybridus* subsp. *quitensis* which are considered different species by some authors (e.g. Xu & Sun 2001). In the *A. hybridus* agg., Townsend (1988) and Stace (1997) consider the names *A. powellii* and *A. hybridus* as taxonomic synonyms. Even if other morphological characters can easily differentiate these taxa, the distinctiveness of their bracteole anatomy supports their separate recognition (Fig. 1 D-E; Table 2, 3). Another taxonomic controversy exists between *A. powellii* and *A. bouchonii* (reviewed by Costea et al. 2001). The morphologic and anatomic differences between their bracteoles support the recognition of *A. bouchonii* as

an infraspecific taxon of *A. powellii* (Costea et al. 2001) (Fig. 1 E–F; Table 2, 3). In *Amaranthus blitum* agg., the three subspecies—subsp. *blitum*, subsp. *oleraceus* and subsp. *emarginatus* are dissimilar with respect to anatomy of the bracteoles:

1. Mid-vein of (foliaceous) bracteoles branched _____ **A. blitum** subsp. **oleraceus** (Fig. 2D)
1. Mid-vein of bracteoles unbranched.
 2. Bracteoles foliaceous (rarely membranous). Cells of the Kranz bundle sheath are separated by the cells of the lower epidermis by a 1–2 layered mesophyll _____ **A. blitum** subsp. **blitum** (Fig. 2C)
 2. Bracteoles membranous. Cells of the Kranz bundle sheath are in contact with the cells of the lower epidermis (mesophyll is absent) _____ **A. blitum** subsp. **emarginatus** (Fig. 2B)

The length of bracteoles and the ratio between bracteole and tepal lengths have been used in the past to classify the infraspecific variability of some *Amaranthus* species (e.g. Thellung 1914; Brenan 1961). For example, the individuals with short bracteoles (equaling the flower perianth) were designated as var. *delilei* (Rich. & Lor.) Thell. in *A. retroflexus*. Such forms are not encountered at the population level, presumably because the short bracteole type is selected against. Based on our current knowledge, such plants cannot even be considered ecophenes, because the shortness of bracteoles can hardly be associated with any ecological advantage. In contrast, the morphology of the bracteoles is one of the few characters that differentiate the grain amaranths (especially *Amaranthus caudatus* and *A. cruentus*) from their wild relatives *A. hybridus* (incl. *A. quitensis*) and *A. powellii*. Thus, the spinose bracteoles of grain amaranths are shorter and thinner compared to those in their wild relatives. This may seem a contradiction with the previous conclusion, but in this particular case the morphology of the bracteoles is important. Domestication of grain amaranths goes back in time more than 7000 years (Sauer 1993), and this character—even if quantitative—is probably genetically fixed. The shorter and softer-spined bracteoles were selected for under domestication because they facilitate harvesting. The survey of many herbarium specimens revealed that cultivated grain amaranths consistently have short bracteoles. Only some specimens of *A. hypochondriacus* may exhibit long bracteoles (comparable with those of *A. powellii*), similarly as in *A. retroflexus* some individuals may have short bracteoles. The distinctiveness of grain amaranths is also supported by morphological (Costea et al. 2001), anatomical (Costea & DeMason 2001), cytological (reviewed by Greizerstein et al. 1997) and molecular data (Xu & Sun 2001).

Bracteoles versus foliage leaves in Amaranthus.—In many plants (e.g., *Chrysosplenium* spp., *Euphorbia* spp., *Melampyrum* spp., Costea unpublished) a continuous morphological and structural transition occurs between foliage leaves and bracts on the same plant. In amaranths, such a transition does not exist. Bracteoles in amaranths are sharply differentiated from the leaves. Several tissues of bracteoles show a reductive tendency. Bracteoles have fewer stomata,

their mesophyll and vascular tissue are less developed, and the Kranz bundle sheath is incomplete. In addition to this developmental abbreviation, formation of new structures without a counterpart in leaves occurs. An example is the sclerenchyma that is absent in *Amaranthus* leaves (e.g. Fisher & Evert 1982; Viana 1993; Esparza-Sandoval et al. 1996), but is an important tissue in the bracteoles. Marginal-meristem activity is intense, giving rise to the one-layered membranous wings, which are also absent from the leaves.

Based on their structure, *Amaranthus* spp. leaves belong to a variant of the Atriplicoid type, having a continuous Kranz sheath around the bundles (reviewed by Dengler & Nelson 1999). However, in the bracteoles, the organization of the Kranz cells in arcs is entirely different from this type, and it calls to mind other C₄ leaf structures described in *Chenopodiaceae* such as the kochioid and solsoid types (reviewed by Dengler & Nelson 1999; Jacobs et al. 2001). Since bracteoles are ontogenetically abbreviated leaves, one cannot refrain from speculating on the old idea that “ontogeny recapitulates phylogeny” (See Gould 1977). It is a fact that *Amaranthus* shares close morphological, structural (Costea & DeMason 2001) and molecular affinities with *Chenopodiaceae* (reviewed by Cuénoud et al. 2002) and these new data provide additional evidence in this respect.

Functions of bracteoles in amaranths.—Two functions can be inferred from the structure of bracteoles in *Amaranthus*: photosynthesis and protection. Bracts (in general) are metamorphosed leaves. Although the degree of modification varies in different genera and families, the other functions of the bracts are subsequently added to the primordial role of photosynthesis. When present, bracts may participate in the development of flowers, fruits and seeds. For example, the contribution of bracts to the achene maturation in *Carpinus* spp. (Hori & Tsuge 1993) or to the development of buds in *Gossypium* spp. (Zhao & Oosterhuis 1999) was found to be significant. Studies made on *Gossypium* spp. showed that the removal of bracts reduced the cotton-boll size and the number of seeds per boll (e.g. Bangal et al. 1985; Ahmed 1994). Similar results—a lower seed yield—were reported after removing the phyllaries from the antodia of *Carthamus tinctorius* and *Helianthus annuus* (e.g. Dhopte & Lall 1981; Hayashi & Hanada 1986). In *Amaranthus*, due to the huge number of flowers that develop in the inflorescences, the photosynthetic role of bracteoles should not be underestimated. A single plant of *Amaranthus retroflexus* can produce approximately 1 000 000 seeds (Hanf 1983). Assuming that the number of female flowers in the inflorescence is at least equal to the number of produced seeds, this would mean there are at least 2 million bracteoles in one inflorescence. Therefore, photosynthesis in bracteoles may significantly contribute to the development of the enormous number of small seeds and consequently to the ecological success of amaranths, especially as weeds. However, this hypothesis needs to be verified and the contribution of bracteoles to total photosynthesis to be established.

In many plants, protection is the most common function acquired by bracts,

which is performed in a multitude of ways, against a multitude of factors. Protection can be limited to the inflorescence and flowers, or it can also be extended to the developing fruits as well. In *Heliconia* the liquid produced in the bracts contributes to the protection against herbivores (Wooton & Sun 1990). Association with ants as a result of extrafloral nectaries developed on bracts provides a defense mechanism against a wide variety of predators (e.g. Scott 1981; McLain 1983). In *Rheum nobile*, an alpine plant, bracts absorb UV radiation and keep the reproductive organs warm (Omori et al. 2000). Mechanical protection of both flowers and fruits against herbivores is obvious for the spinose bracteoles in *Amaranthus*. There seems to be a correlation between the presence of spinose bracteoles and the dehiscence of fruits: species with dehiscent fruits have spinose bracteoles, while species with indehiscent fruits have membranous or foliaceous bracteoles (although a few exceptions exist). One of these exceptions is *A. powellii* subsp. *bouchonii* that has indehiscent fruits and spinose bracteoles. Yet, this taxon has shorter and thinner bracts compared to *A. powellii* subsp. *powellii*, its closest relative with circumscissile fruits (Costea et al. 2001). Furthermore, in monoecious and some dioecious species (e.g. *A. palmeri*) the bracteoles of the female flowers are more developed than the bracteoles of the male flowers. The abortive female flowers of hybrids are subtended by longer (than normally) bracteoles (Brenner, unpublished).

In conclusion, based on the populations and accessions examined, bracteoles are important for the species delimitation in *A. hybridus* agg. and for the separation of subspecies in *A. blitum*. The anatomical study of bracteoles, along with other characters (the vascular system and the structure of fruits) indicates that subgenus *Albersia* has a basal phylogeny within *Amaranthus*, while the species of the subgenus *Amaranthus* are derived. Protection and photosynthesis are the main functions deduced from the structure of the bracteoles.

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